Microsphere Mediated Delivery of Differentiation Factors for Directed Embryonic Stem Cell Differentiation

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Statement of Purpose: Stem cell technologies have shown great potential in regenerative medicine applications, yet various limitations have prevented their use clinically, including the inability to induce controllable, homogenous differentiation to targeted cell types. In the developing embryo, signals that direct differentiation are presented in a temporally and spatially controlled manner. The effects of signaling molecules on embryonic stem (ES) cell differentiation in vitro can vary depending on the concentration and timing of introduction to cells. Thus, techniques for presenting signaling molecules to ES cells in a spatially and temporally controlled manner need to be integrated into differentiation methods. The differentiation of ES cells via formation of non-adherent spheroids known as embryoid bodies (EBs) presents additional challenges, as their three-dimensional nature and relatively large size may introduce a barrier for the diffusion of signaling molecules into the interior of EBs to interact with all cells. Degradable polymer microspheres have been employed as both an adherent surface for cells and a controlled release biomolecular delivery vehicle. The objective of this research is to incorporate microspheres within the local microenvironment EBs to increase the homogeneity of biomolecular distribution and achieve more homogeneous cell differentiation.

Methods: Poly(lactic-co-glycolic acid) (50:50, MW 75 kDa) microspheres encapsulating retinoic acid (RA) or fluorescent dye were fabricated using an oil-in-water emulsion, solvent evaporation technique. Microspheres were analyzed with scanning electron microscopy and RA release was quantified spectrophotometrically. Particles coated with gelatin, laminin, and poly(lysine) and uncoated particles were mixed with ES cells in rotary suspension culture during embryoid body formation. Incorporation of labeled microspheres within EBs was assessed using fluorescent microscopy. Gelatin coated RA loaded microspheres were mixed with ES cells in rotary suspension culture, and gene expression and morphology were compared to soluble RA treatment and untreated EBs using quantitative PCR and hematoxylin and eosin (H&E) staining, respectively.

Results / Discussion: Gelatin coated microspheres were incorporated into EBs most efficiently, with nearly 100% of EBs incorporating at least one microsphere, compared to 57% and 30% for poly(lysine) coated and uncoated microspheres, respectively (Figure 1A). Laser scanning confocal microscopy confirmed that microspheres were distributed throughout the interior of EBs and not simply adherent to the exterior cells of EBs. Gene expression analysis revealed that nestin, an intermediate filament protein found in neural progenitor cells (NPCs), was both upregulated and subsequently downregulated earlier in EBs treated with RA loaded microspheres than in untreated or soluble RA treated EBs (Figure 1B). This may indicate an earlier appearance of NPCs followed by accelerated differentiation into more mature phenotypes in RA loaded microsphere treated EBs compared to soluble RA or untreated EBs. Histological examination by H&E staining revealed tight cellular packing in soluble RA treated EBs, indicated by dense hematoxylin-stained regions, whereas untreated EBs appeared more loosely assembled, with more punctate nuclear staining as well as more void space (Figure 1C). EBs incorporating RA loaded microspheres showed punctate nuclei similar to untreated EBs, yet also contained pockets of dense staining similar to EBs treated with RA solubly (red arrows), suggesting RA release from microspheres within EBs locally affected cell differentiation.

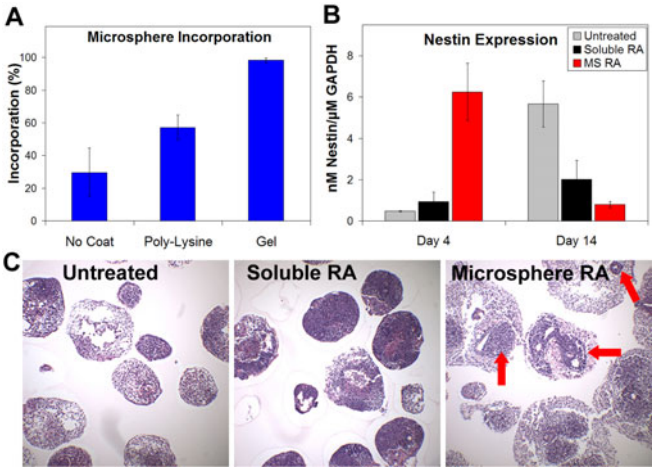

Figure 1. A) Incorporation of microspheres with different coatings into EBs. B) Nestin expression in untreated, soluble RA, and microsphere RA treated EBs. C) H&E staining of EBs. Red arrows indicate pockets of dense staining in microsphere RA EBs.

Conclusions: Polymer microspheres containing differentiation factors can be incorporated within EBs with high efficiency. Preliminary evidence suggests that microsphere-mediated delivery results in localized release of RA within EBs and induces more efficient ES cell differentiation than soluble treatment. The microsphere-mediated delivery approach provides a means to modulate the distribution of signaling factors to induce more homogeneous ES cell differentiation from EBs. Future experiments will focus on quantifying the homogeneity of biomolecular distribution within EBs using confocal microscopy and image analysis software. Additionally, immuno-staining with flow cytometry will be performed to quantitatively assess the effect of controlled biomolecule presentation on ES cell differentiation.